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(54) Title: NOVEL LHRH ANTAGONISTS WITH IMPROVED SOLUBILITY CHARACTERISTICS

(54) Bezeichnung: NEUE LHRH-ANTAGONISTEN MIT VERBESSERTEN LÖSLICHKEITSEIGENSCHAFTEN

(57) Abstract

The invention relates to peptides which contain N-methylated amino acid building blocks and are provided with improved water solubility. Medicaments containing the inventive peptides can be used for the treatment of hormone-dependent tumours and hormone-influenced, non-malignant diseases.

(57) Zusammenfassung

Die Erfindung betrifft Peptide, die N-methylierte Aminosäurebausteine enthalten und eine verbesserte Wasserlöslichkeit aufweisen. Arzneimittel, in denen die erfindungsgemässen Peptide enthalten sind, können zur Behandlung hormonabhängiger Tumore und hormonbeeinflusster nicht-maligner Erkrankungen verwendet werden.

Novel LHRH antagonists having improved solubility properties

The invention relates to LHRH antagonists having improved solubility properties, processes for the preparation of these compounds, medicaments in which these compounds are contained, and the use of the medicaments for the treatment of hormone-dependent tumours and hormone-influenced non-malignant disorders such as benign prostate hyperplasia (BPH) and endometriosis.

The nomenclature used for the definition of the peptides agrees with that nomenclature explained by the IUPAC-IUB Commission on Biochemical Nomenclature (European J. Biochem. 1984, 138, 9-37), in which, in agreement with the conventional representation, the amino groups at the N terminus appear to the left and the carboxyl group at the C terminus appears to the right. The LH-RH antagonists such as the peptides according to the invention include naturally occurring and synthetic amino acids, the former including Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met, Phe, Tyr, Pro, Trp and His. The abbreviations for the individual amino acid residues are based on the trivial names of the amino acids and are Ala=alanine, Arg=arginine, Gly=glycine, Leu=leucine, Lys=lysine, Pal(3)=3-(3-pyridyl)alanine, Nal(2)=3-(2-naphthyl)-alanine, Phe=phenylalanine, Cpa=4-chlorophenylalanine, Pro=proline, Ser=serine, Thr=threonine, Trp=tryptophan, Try=tyrosine and Sar=sarcosine. All amino acids described here originate from the L series, if not mentioned otherwise. For example, D-Nal(2) is the abbreviation for 3-(2-naphthyl)-D-alanine and Ser is the abbreviation for L-serine. Substitutions on the ε amino group in the side chain of lysine are represented by a term placed in brackets behind Lys, if appropriate in the form of an abbreviation.

The first series of potent antagonists was obtained by the introduction of aromatic amino acid residues into the positions 1, 2, 3 and 6 or 2, 3 and 6. The customary way of writing the compounds is as follows: the amino acids are first indicated which have taken the place of the amino acids originally present in the peptide chain of LH-RH, the positions in which the exchange took place being marked by superscripted figures. Furthermore, by the notation "LH-RH" placed afterwards it is expressed that these are LH-RH analogues in which the exchange has taken place.

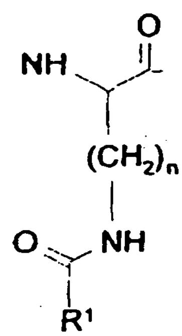
Known antagonists are:
[Ac-D-Cpa^{1,2}, D-Trp^{3,6}] LH-RH (D.H. Coy et al., In: Gross, E. and Meienhofer, J. (Eds) Peptides; Proceedings of the 6th American Peptide Symposium, pp. 775-779, Pierce Chem. Co., Rockville III. (1979): [Ac-Pro^{1}, D-Cpa^{2}, D-Nal(2)^{3,6}] LH-RH (US Patent No. 4,419,347) and [Ac-Pro^{1}, D-Cpa^{2}, D-Trp^{3,6}] LH-RH (J.L. Pineda, et al., J. Clin. Endocrinol. Metab. 56, 420, 1983).

In order to improve the action of antagonists, basic amino acids, for example D-Arg, were later introduced into the 6 position. For example [Ac-D-Cpa^{1,2}, D-Trp^{3}, D-Arg^{6}, D-Ala^{10}] LH-RH (ORG-30276) (D.H. Coy, et al., Endocrinology 100, 1445, 1982); and [Ac-D-Nal(2)1, D-Phe(4-F)^{2}, D-Trp^{3}, D-Arg^{6}] LH-RH (ORF 18260) (J.E. Rivier et al., in: Vickery B.H. Nestor, Jr. J.J., Hafez, E.S.E (Eds). LHRH and its Analogs, pp. 11-22 MTP Press, Lancaster, UK 1984).

Further potent LH-RH antagonists are described in WO 92/19651, WO 94/19370, WO 92/17025, WO 94/14841, WO 94/13313, US-A 5,300,492, US-A 5,140,009, EP 0 413 209 A1 and DE 195 44 212 A1.

A-Xxx^1-Xxx^2-Xxx^3-Xxx^4-Xxx^5-Xxx^6-Xxx^7-Xxx^8-Xxx^9-Xxx^{10}-NH_2
(I)

in which
A is an acetyl or a 3-(4-fluorophenyl)propionyl group,
Xxx^1 is D-Nal(1) or D-Nal(2),
Xxx^2-Xxx^3 is D-Cpa-D-Pal(3) or a single bond,
Xxx^4 is Ser,
Xxx^5 is N-Me-Tyr,
Xxx^6 is D-Cit, D-Hci or a D-amino acid group of the general formula (II)

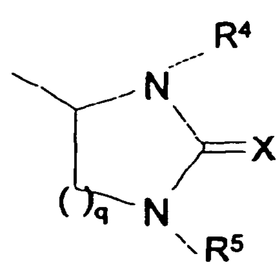


(II)

in which n is the number 3 or 4, where R^1 is a group having the general formula III

$-(CH_2)_p$-CO-NR^2R^3 (III)

where p is an integer from 1 to 4, R^2 is hydrogen or an alkyl group and R^3 is an unsubstituted or substituted aryl group or heteroaryl group, or R^1 is a 3-amino-1,2,4-triazole-5-carbonyl group or R^1 is a ring of the general formula (IV)



(IV)

The compounds according to the invention can be used for the treatment of hormone-dependent tumours, in particular prostate carcinoma or breast cancer, and also for non-malignant indications whose treatment necessitates LH-RH hormone suppression. For this, they are mixed with the customary vehicles and excipients and formulated as medicaments.

The synthesis of compounds according to formula (I) can both be carried out either by classical fragment condensation or by solid-phase synthesis according to Merrifield with synthesis following one another using D-lysine already acylated in the side chain with the carboxylic acid of the general formula R^1-COOH or by reaction of a decapeptide unit with the appropriate carboxylic acids by amide linkage in the side chain of D-lysine^6. Accordingly, the introduction of the R^1-CO- group can be performed in three different positions in the process: before the condensation of the individual units to give the peptide, after the incorporation of lysine or ornithine in the peptide chain, but before the condensation of the next unit or after condensation of all units.

The compounds of the formula (I) are synthesized according to the known methods, such as, for example, by pure solid-phase technique, partly solid-phase technique (so-called fragment condensation) or by the classical solution couplings (see M. Bodanszky, "Principles of Peptide Synthesis", Springer Verlag 1984).
For example, the methods of solid-phase synthesis are described in the textbook "Solid Phase Peptide Synthesis", J.M. Stewart and J.D. Young, Pierce Chem. Company, Rockford, III, 1984, and in G. Barany and R.B. Merrifield "The Peptides", Ch. 1, pp. 1-285, 1979, Academic Press Inc. Classical solution syntheses are

Esters of N-protected amino acids, such as, for example, N-hydroxysuccinimide esters or 2,4,5-trichlorophenyl esters, are particularly highly suitable for the stepwise condensation of amino acids. The aminolysis can be very well catalysed by N-hydroxy compounds which have approximately the acidity of acetic acid, such as, for example, 1-hydroxybenzotriazole.

Intermediate amino protective groups which present themselves are groups which are removed by hydrogenation, such as, for example, the benzyloxycarbonyl radical (= Z radical) or groups which can be removed by weak acid. Suitable protective groups for the α-amino groups are, for example:
tertiary butyloxycarbonyl groups, fluorenylmethyl-oxycarbonyl groups, carbobenzoxy groups or carbobenzothio groups (if appropriate in each case having a p-bromo [sic] or p-nitrobenzyl radical), the trifluoroacetyl group, the phthalyl radical, the o-nitrophenoxyacetyl group, the trityl group, the p-toluenesulphonyl group, the benzyl group, benzyl radicals substituted in the benzene nucleus (p-bromo [sic] or p-nitrobenzyl radical) and the α-phenylethyl radical. Reference is also made here to P. Greenstein and Milton Winitz, Chemistry of Amino Acids, New York 1961, John Wiley and Sons, Inc., Volume 2, for example page 883 et seq., "Principles of Ppetide Synthesis", Springer Verlag 1984, "Solid Phase Peptide Synthesis", J.M. Stewart and J.D. Young, Pierce Chem. Company, Rockford, III, 1984, G. Barany and R.B. Merrifield "The Peptides", Ch. 1, pp. 1-285, 1979, Academic Press Inc., and also The Peptides, Volume 2, Ed. E. Gross and J. Maienhofer, Academic Press, New York. These protective groups are fundamentally also suitable for the protection of further functional side groups (OH groups, NH_2 groups) of the corresponding amino acids.

swollen in organic solvents (for example a copolymer of polystyrene and 1% divinylbenzene). The synthesis of a protected decapeptide amide on a methylbenzhydrylamide [sic] resin (MBHA resin, i.e. polystyrene resin provided with methylbenzhydrylamide [sic] groups), which affords the desired C-terminal amide function of the peptide after HF cleavage from the support, can be carried out according to the following flow diagram:

<u>Flow diagram</u>

Peptide synthesis protocol

Stage	Function	Solvent/Reagent (v/v)	Time
1	Washing	Methanol	2 × 2 min
2	Washing	DCM	3 × 3 min
3	Removal	DCM/TFA (1:1)	1 × 30 min
4	Washing	Isopropanol	2 × 2 min
5	Washing	Methanol	2 × 2 min
6	Washing	DCM	2 × 3 min
7	Neutralization	DCM/DIPEA (9:1)	3 × 5 min
8	Washing	Methanol	2 × 2 min
9	Washing	DCM	3 × 3 min
10	STOP	Addition of the Boc-As in DCM + DIC + HOBt	
11	Coupling	DCM, optionally DCM/DCF	approx. 90 min
12	Washing	Methanol	3 × 2 min
13	Washing	DCM	2 × 3 min

The Nα-Boc-protected amino acids are customarily coupled in a three fold molar excess in the presence of diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in CH_2Cl_2/DMF in the course of 90 min, and the Boc-protected group is removed by action of 50% trifluoroacetic acid (TFA) in CH_2Cl_2 for half an hour. To check for complete conversion, the chloranil test according to Christensen and the Kaiser's ninhydrin test can be used. Radicals of free amino function [sic]

The following examples serve to illustrate the invention without restricting it.

Example 1

Ac-D-Nal(2)^{1}-D-Cpa^{2}-D-Pal(3)^{3}-Ser^{4}-N-Me-Tyr^{5}-D-Hci^{6}-Nle^{7}-Arg^{8}-Pro^{9}-D-Ala^{10}-NH_2

The synthesis was carried out according to a solid-phase flow diagram (Peptide Synthesis Protocol, p. 11) with DIC/HOBt coupling, starting from 3.3 g of MBHA resin (loading density 1.08 mmol/g). After HF cleavage from the polymeric support, 3.4 g of crude peptide were obtained, which were purified by standard processes of preparative HPCI [sic]. After subsequent freeze-drying, 1.43 g of HPLC-uniform product of the empirical formula C72, H96, N17, O14, Cl [sic] having correct FAB-MS: 1458.7 $(M+H^+)$ (calc: 1457.7), and corresponding ^{1}H-NMR spectrum were obtained.

^{1}H-NMR (500 MHz, D_2O/DMSO-d_6, δ in ppm):
8.7 to 7.2, several m, arom. H and incompletely exchanged NH; 6.92 and 6.58, 2d, 2×2H, arom. H p-Cl-Phe; 5.2 to 3.5, several m, Cα-H and aliph. H; 3.2 to 2.6, several m, aromat. Cβ-H 2.1 to 0.7, several m, residual aliphat. H; 1.70, s, 3H, acetyl; 1.20, d, 3H, Cβ-H Ala; 0.8, m, Cδ-H Leu

Example 2

Ac-D-Nal(2)^{1}-D-Cpa^{2}-D-Pal(3)^{3}-Ser^{4}-N-Me-Tyr^{5}-D-Lys(B)^{6}-Leu^{7}-Lys(iPr)^{8}-Pro^{9}-D-Ala^{10}-NH_2

The synthesis was carried out according to a flow diagram (Peptide Synthesis Protocol, p. 11) with DIC/HOBt coupling, starting from 4.0 g of MBHA resin (loading density 1.11 mmol/g). After HF cleavage from the polymeric support, 4.87 g of crude peptide were

empirical formula C82, H106, N19, O15, Cl [sic] having correct ESI-MS: 1632.7 $(M+H^+)$ (calc: 1631.7), and corresponding ^1H-NMR spectrum were obtained.

^1H-NMR (500 MHz, DMSO-d_6, δ in ppm):
10.4, s, 1H and 9.15, s, 2H, and 9.0, s, 2H, NHs of 4-amidinoaniline; 8.60, m, 2H, arom. H; 8.3 to 7.2, several m, arom. H and NH; 7.27 and 7.20, 2d, 4H, arom. H (pCl)Phe; 6.96 and 6.60, 2d, 4H, arom. H Tyr; 5.2 to 3.5, several m, Cα-H and aliphat. H; 3.2 to 2.4, several m, Cβ-H and $N-CH_3$; 2.1 to 1.1, several m, residual aliphat. H; 1.70, s, 3H, acetyl; 1.20, d, 3H, Cβ-H Ala; 0.85, dd, 6H, Cδ-H Leu

<u>Example 4</u>

Ac-D-Nal(2)^1-D-Cpa^2-D-Pal(3)^3-Ser^4-N-Me-Tyr^5-D-Hci^6-Nle^7-Lys(iPr)^8-Pro^9-D-Ala^{10}-NH_2

The synthesis was carried out according to a solid-phase flow diagram (Peptide Synthesis Protocol, p. 11) with DIC/HOBt coupling, starting from 2.5 g of MBHA resin (loading density 1.08 mmol/g). After HF cleavage from the polymeric support, 2.78 g of crude peptide were obtained, which were purified by standard processes of preparative HPCI [sic]. After subsequent freeze-drying, 400 mg of HPLC-uniform product of the empirical formula C75, H102, N15, O14, Cl [sic] having correct ESI-MS: 1472.6 $(M+H^+)$ (calc: 1471.7), and corresponding ^1H-NMR spectrum were obtained.

^1H-NMR (500 MHz, D_2O/DMSO-d_6, δ in ppm):
8.62, m, 2H, 8.30, m, 2H, 7.80, m, 4H, 7.66, s, 1H, 7.47, m, 2H, 7.36, d, 1H, aromat. H; 7.25 and 7.20, 2 d, 4H, arom. H (pCl)Phe; 6.96 and 6.63, 2d, 4H, aromat. H Tyr; 5.10 to 4.0, several m, Cα-H and aliphat. H; 3.75 to 2.65, several m, Cβ-H and $N-CH_3$; 2.1 to 1.05, several m, residual aliphat. H; 1.74, s,

Example 6

3-(4-Fluorophenyl)propionyl-D-Nal(1)^{1}-Ser^{4}-N-Me-Tyr^{5}-D-Lys(Atz)^{6}-Leu^{7}-Arg^{8}-Pro^{9}-D-Ala^{10}-NH_2

The synthesis was carried out according to a solid-phase flow diagram (Peptide Synthesis Protocol, p. 11) with DIC/HOBt coupling, starting from 9.2 g of MBHA resin (loading density 1.08 mmol/g). After HF cleavage from the polymeric support, 5.8 g of crude peptide were obtained, which were purified by standard processes of preparative HPCI [sic]. After subsequent freeze-drying, 2.0 g of HPLC-uniform unsubstituted octapeptide were obtained, of which 0.4 mmol was reacted with 0.5 mmol of 3-amino-1,2,4-trizole-5-carboxylic acid in the presence of PyBOP as a coupling reagent to give 790 mg of crude product of the desired compound. After fresh HPLC purification, 200 mg of target compound of the empirical formula C64, H86, N17, O12, F [sic] having correct FAB-MS: 1304.6 ($M+H^+$) (calc: 1303.6) were obtained.

^{1}H-NMR (500 MHz, D_2O/DMSO-d_6, δ in ppm):
8.14, m, 1H, 7.90, m, 1H, 7.80, m, 1H, 7.50, m, 2H, 7.35, m, 2H, 7.0, m, 6H, 7.63, m, 2H, aromat. H; 5.0, m, 1H, 4.83, m, 2H, 4.41, m, 1H, 4.30 - 4.05, several m, 4H, Cα-H; 3.66 to 2.25, several m, aliphat. and aromat. side-chain H; 2.95, s, and 2.75, s, N-Me; 2.05 to 1.1, several m, residual aliphat. H; 1.20, d, Cβ-H Ala; 0.75, m, 6H, Cδ-H Leu

The compounds according to formula I according to the invention were investigated for their receptor binding. The process closely followed the process described in Beckers et al., Eur. J. Biochem. 231, 535-543 (1995). Cetrorelix obtained according to the synthesis disclosed above was iodinated with [^{125}I] (Amersham; specific activity 80.5 Bq/fmol) using the IodoGen

$NaCl/P_i$ (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 11.47 mM KH_2PO_4)/1 mM EDTA and collected by centrifugation. The cell pellet was resuspended in binding buffer (DMEM without H_2CO_3, with 4.5 g/l of glucose, 10 mM Hepes pH 7.5, 0.5% (mass/volume) BSA, 1 g/l bacitracin, 0.1 g/l SBTI, 0.1% (mass/volume) NaN_3). For displacement assays, 0.25×10^6 cells/100 µl were incubated with approximately 225 pM of the [^{125}I]-cetrorelix (specific activity $5\text{-}10 \times 10^5$ dpm/pmol) and various concentrations of unlabelled compound according to the invention as competitor. The cell suspension in 100 µl of binding medium was layered in 400 µl assay tubes over 200 µl of 84% by volume silicone oil (Merck Type 550)/16% by volume paraffin oil. After incubation for 1 h at 37°C with slow, continuous shaking, the cells were separated from the incubation medium by centrifugation for 2 min at 9000 rpm (rotor type HTA13.8; Heraeus Sepatec, Osterode/Germany). The tips of the tubes which contained the cell pellet were cut off. Cell pellet and supernatants were then analysed by counting the γ radiation. The amount of non-specifically bound material was determined at a final concentration of 1 µM with inclusion of unlabelled cetrorelix and was typically ≤ 10% of the total bound material. The analysis of the binding data was carried out using the EBDA/ligand analysis programme (Biosoft V3.0).

Method 2.

Functional assay for the determination of the antagonistic activity

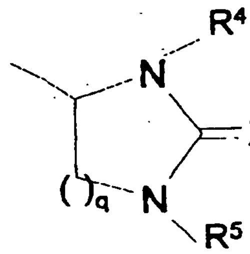
The assay was carried out, provided with some modifications, as described in Beckers, T., Reiländer, H., Hilgard, P. (1997) "Characterization of gonadotropin-releasing hormone analogs based on a sensitive cellular luciferase reporter gene assay",

In this way, the following in-vitro data were obtained, K_D being the binding affinities and IC_{50} being the functional activity and pM being picomoles per litre:

Compound	K_D [pM]	IC_{50} [pM]
cetrorelix	170 (21)	198 (5)
Example 1 (Acetate salt)	n.d.	242 (3)
Example 2	181 (1)	684 (2)
Example 3	154 (1)	492 (2)
Example 6	n.d.	221 (2)

n.d. = not determined

() = number of independent experiments



(IV)

in which q is the number 1 or 2, R^4 is a hydrogen atom or an alkyl group, R^5 is a hydrogen atom or an alkyl group and X is an oxygen or sulphur atom,
Xxx^7 is Leu or Nle,
Xxx^8 is Arg or Lys(iPr),
Xxx^9 is Pro and
Xxx^{10} is Ala or Sar,
and their salts with pharmaceutically acceptable acids.

2. Compounds according to Claim 1, in which the salt is an acetate, trifluoroacetate or embonate.

3. Compounds according to Claim 1 or 2, in which Xxx^6 is D-[ε-N'-(imidazolidin-2-on-4-yl)formyl]-Lys, D-(3-amino-1,2,4-triazole-3-carbonyl)-Lys, abbreviated D-Lys(Atz) or D-[ε-N'-4-(4-amidino-phenyl)amino-1,4-dioxobutyl]-Lys, abbreviated D-Lys(B).

4. Compound according to Claim 1 having the formula:
Ac-D-Nal(2)^1-D-Cpa^2-D-Pal(3)^3-Ser^4-N-Me-Tyr^5-D-Hci^6-Nle^7-Arg^8-Pro^9-D-Ala^{10}-NH_2.

5. Compound according to Claim 1 having the formula:
Ac-D-Nal(2)^1-D-Cpa^2-D-Pal(3)^3-Ser^4-N-Me-Tyr^5-D-Lys(Atz)^6-Leu^7-Arg^8-Pro^9-D-Ala^{10}-NH_2.

6. Compound according to Claim 1 having the formula:
Ac-D-Nal(2)^1-D-Cpa^2-D-Pal(3)^3-Ser^4-N-Me-Tyr^5-D-Lys(B)^6-Leu^7-Lys(iPr)^8-Pro^9-D-Ala^{10}-NH_2.

14. Use of the substances according to Claims 1 to 11 for producing medicaments for the treatment of hormone-dependent tumours, in particular prostate carcinoma or breast cancer, and also for non-malignant indications whose treatment necessitates LH-RH hormone suppression.

15. Process for producing medicaments, in which compounds according to Claims 1 to 11 are mixed with the customary vehicles and excipients and formulated as medicaments.